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Aggravating Impact of Nanoparticles on Immune-Mediated Pulmonary Inflammation

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Although the adverse health effects of nanoparticles have been proposed and are being clarified, their aggravating effects on pre-existing pathological conditions have not been fully investigated. In this review, we provide insights into the immunotoxicity of both airborne and engineered nanoparticles as an exacerbating factor on hypersusceptible subjects, especially those with immune-mediated pulmonary inflammation, using our *in vivo* experimental model. First, we exhibit the effects of nanoparticles on pulmonary inflammation induced by bacterial endotoxin (lipopolysaccharide: LPS) as a disease model in innate immunity, and demonstrate that nanoparticles instilled through both an intratracheal tube and an inhalation system can exacerbate the lung inflammation. Second, we introduce the effects of nanoparticles on allergic pulmonary inflammation as a disease model in adaptive immunity, and show that repetitive pulmonary exposure to nanoparticles has aggravating effects on allergic inflammation, including adjuvant effects on Th2-milieu. Third, we show that very small nanoparticle exposure exacerbates emphysematous pulmonary inflammation, which is concomitant with enhanced lung expression of proinflammatory molecules (including those that are innate immunity related). Taken together, nanoparticle exposure may synergistically facilitate pathological pulmonary inflammation via both innate and adaptive immunological impairment.

KEYWORDS: nanoparticles, immune-mediated pulmonary inflammation, lipopolysaccharide, ovalbumin, elastase

INTRODUCTION

Epidemiological studies have demonstrated a correlation between exposure to air pollutant particles at the concentrations currently found in major metropolitan areas and mortality and morbidity[1]. The concentration of particulate matter (PM) with a mass median aerodynamic diameter (a density-dependent unit of measure used to describe the diameter of the particle) ≤2.5 μm ($PM_{2.5}$) is more closely associated with both acute and chronic respiratory effects and subsequent mortality than larger particles of ≤10 μm (PM_{10})[2]. In addition, one intriguing aspect of the epidemiologic data is that health effects of $PM_{2.5}$ are primarily seen in subjects with predisposing factors, including pneumonia, asthma, chronic obstructive

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pulmonary disease, compromised immune systems, atherosclerosis, age over 65 years, and possible depressive state[3,4,5,6]. Partially consistent with the epidemiological studies, we and others have experimentally demonstrated that diesel exhaust particles (DEP), major contributors to environmental $PM_{2.5}$ in urban areas, exhibit respiratory toxicity with or without predisposing pathologies, including allergic asthma, pulmonary emphysema, and acute renal failure (although we did not provide evidence that DEP significantly exacerbated a murine model of pulmonary emphysema in our system[7]) *in vivo*[8,9,10,11,12,13,14,15,16].

To date, nanoparticles, particles <0.1 μm in mass median aerodynamic diameter, have been shown to be increasing in ambient air[17]. Recent measurements indicate that nanoparticle numbers in ambient air range from $2 \times 10^4/cm^3$ to $2 \times 10^5/cm^3$, with mass concentrations of >50 $\mu g/m^3$ near major highways[18,19]. On the other hand, nanotechnology is now advancing at an incredible pace, such that it is has created an alternative industrial revolution over the past few years[20]. Consistent with this, the use of engineered nanoparticles (called as "nanomaterials") has been rapidly increasing in commercial applications. As these particles/materials have become more widespread, many questions have arisen regarding the adverse effects they may have on the environment as alternative inhalable toxicants. In particular, due to their sizes, characteristics, and/or existing pattern, nanoparticles/nanomaterials have been implicated in cardiopulmonary system effects[21]. Furthermore, compared to larger particles, nanoparticles/nanomaterials have a higher deposition rate in the peripherial lung, they can cross the pulmonary epithelium and reach the interstitium[22], and may thus be systemically distributed in the bloodstream[23], raising the possibility to render larger degree of inflammation. Nanoparticle/ nanomaterials have an enhanced capacity to produce reactive oxygen species (ROS) and, consequently, have a widespread toxicity[24,25,26], as do other types of PM (with relatively larger particle size), since ROS generation by particles can exert protein, lipid, and membrane damage[27,28]. For example, transition metals and redox-cycling organic chemical components on the particle surface can also participate in ROS generation characterized by the formation of O_2^- through dismutation or Fenton reaction[28]. Indeed, it has been reported that nanomaterial exposure itself induces lung inflammation as for carbon nanotube and titanium dioxide, gold, and quantum dot[29,30,31,32,33,34]. These particle/material exposures also reportedly influence/promote cardiopulmonary systems in the presence of predisposing diseases in human studies[35,36]. However, biological evidence concerning the promoting effects of nanoparticles on predisposing subjects has been less studied. Besides their toxic property on health, therefore, it should be experimentally ascertained whether they also aggravate pre-existing pathological conditions and their underlying mechanisms should be resolved. In this review, we will discuss the impact of nanoparticles as immunological enhancers in the respiratory system.

EFFECTS OF NANOPARTICLES ON BACTERIAL ENDOTOXIN-ELICITED PULMONARY INFLAMMATION

A glycolipid of Gram-negative bacteria, known as endotoxin or lipopolysaccharide (LPS), stimulates host cells via innate immunity[37]. In animal models, intratracheal administration of LPS causes lung cytokine expression, neutrophil recruitment, and lung injury[38]. LPS is found in the bronchoalveolar lavage (BAL) fluid of patients with pneumonia[39] and acute respiratory distress syndrome[40], which sometimes results in a fatal outcome. In addition, LPS is a significant constituent of many air pollutant particles and has accordingly been implicated in the adverse effects of PM[41]. In accordance with the close links among LPS, lung inflammation (injury), and PM, we have previously shown that intratracheal administration of DEP and their components facilitates lung inflammation induced by LPS[14,42].

We previously examined the effects of pulmonary exposure to nanoparticles on lung inflammation related to LPS in mice. Vehicle, two different types (particle size: 14 and 56 nm) of carbon black nanoparticles (CBNP), LPS, or LPS + CBNP was administered intratracheally, and parameters of lung inflammation and coagulation were evaluated. CBNP alone induced slight lung inflammation and significant pulmonary edema as compared with the vehicle. The 14-nm CBNP intensively aggravated

LPS-elicited lung inflammation and pulmonary edema, which was concomitant with the enhanced lung expression of interleukin (IL)-1β, macrophage inflammatory protein (MIP)-1α, macrophage chemoattractant protein (MCP)-1, MIP-2, and keratinocyte chemoattractant (KC) in overall trend, whereas the 56-nm CBNP did not show apparent effects. Immunoreactivity for 8-hydroxyguanosine (OHdG), a proper marker for oxidative stress, was more intense in the lung from the LPS + 14-nm CBNP group than in that from the LPS group. Taken together, CBNP can aggravate lung inflammation related to bacterial endotoxin, which is more prominent with smaller particles. The enhancing effect may be mediated, at least partly, via the increased local expression of proinflammatory cytokines and via the oxidative stress[43].

Furthermore, we examined the adverse effects of engineered nanoparticles on this pathological model. In brief, Institute for Cancer Research (ICR) male mice were divided into eight experimental groups that intratracheally received vehicle, three sizes (15, 50, or 100 nm) of TiO_2 nanoparticles, LPS, or LPS + TiO_2 nanoparticles. Twenty-four hours after the treatment, this type of nanoparticle exacerbated the lung inflammation and edema elicited by LPS, with an overall trend of amplified lung expressions of cytokines, such as IL-1β, MCP-1, and KC. LPS + nanoparticles, especially with size <50 nm, elevated circulatory levels of IL-1β, MCP-1, and KC as compared with LPS alone. The enhancement tended overall to be greater with the smaller nanoparticles than with the larger ones. These results suggest that engineered nanoparticles also exacerbate lung inflammation related to LPS and accompanying systemic inflammation, and the exacerbation is more prominent with smaller nanoparticles than with larger particles[44]. Additionally, we demonstrated that latex nanoparticles[45] and carbon nanotubes[46] have similar aggravating potential on the lung pathophysiology.

Our next study was conducted to determine whether inhaled exposure to diesel engine–derived nanoparticles also exacerbates the model. ICR mice were exposed for 5 h to clean air or diesel engine–derived nanoparticles at a concentration of 15, 36, or 169 $\mu g/m^3$ after intratracheal challenge with LPS or vehicle, and were sacrificed for evaluation 24 h after the intratracheal challenge. Exposure to nanoparticles alone did not elicit lung inflammation. Nanoparticle inhalation exaggerated LPS-elicited inflammatory cell recruitment in the BAL fluid and lung parenchyma as compared with clean air inhalation in a concentration-dependent manner. Lung homogenates derived from the LPS + nanoparticle groups tended to have an increased tumor necrosis factor-α level and chemotaxis activity for polymorphonuclear leukocytes as compared to those from the LPS group or the corresponding nanoparticle groups. Nanoparticle inhalation did not significantly increase the lung expression of proinflammatory cytokines or influence systemic inflammation. Isolated alveolar macrophages from nanoparticle-exposed mice showed a greater production of IL-1β and KC stimulated with *ex vivo* LPS challenge than those from clean air–exposed mice, although the differences did not reach significance. These results suggest that acute exposure to diesel nanoparticles exacerbates lung inflammation induced by LPS[47]. In sum, nanoparticle exposure exacerbates acute lung inflammation related to bacterial endotoxin (Fig. 1).

EFFECTS OF NANOPARTICLES ON ALLERGEN-ELICITED PULMONARY INFLAMMATION

Bronchial asthma has been recognized as chronic airway inflammation with hyper-responsiveness that is characterized by an increase in the number of activated lymphocytes and eosinophils[48]. A number of studies have shown that various particles, including carbon black, can enhance allergic sensitization[49,50,51], which is referred to as "adjuvant effect". As well, carbon black has been demonstrated to enhance the proliferation of antibody-forming cells and both IgE and IgG levels[52,53]. Ultrafine particles (PM and carbon black) reportedly exaggerate allergic airway inflammation *in vivo*[54,55]. However, all studies have failed to pay attention to the size of the particles. Therefore, no research has addressed the size effects of particles or nanoparticles on airway biology in the presence or absence of allergen *in vivo*. Given the hypothesis, we investigated the effects of CBNP with a diameter of 14 or 56 nm on allergen-related airway inflammation. ICR mice were divided into six experimental groups.

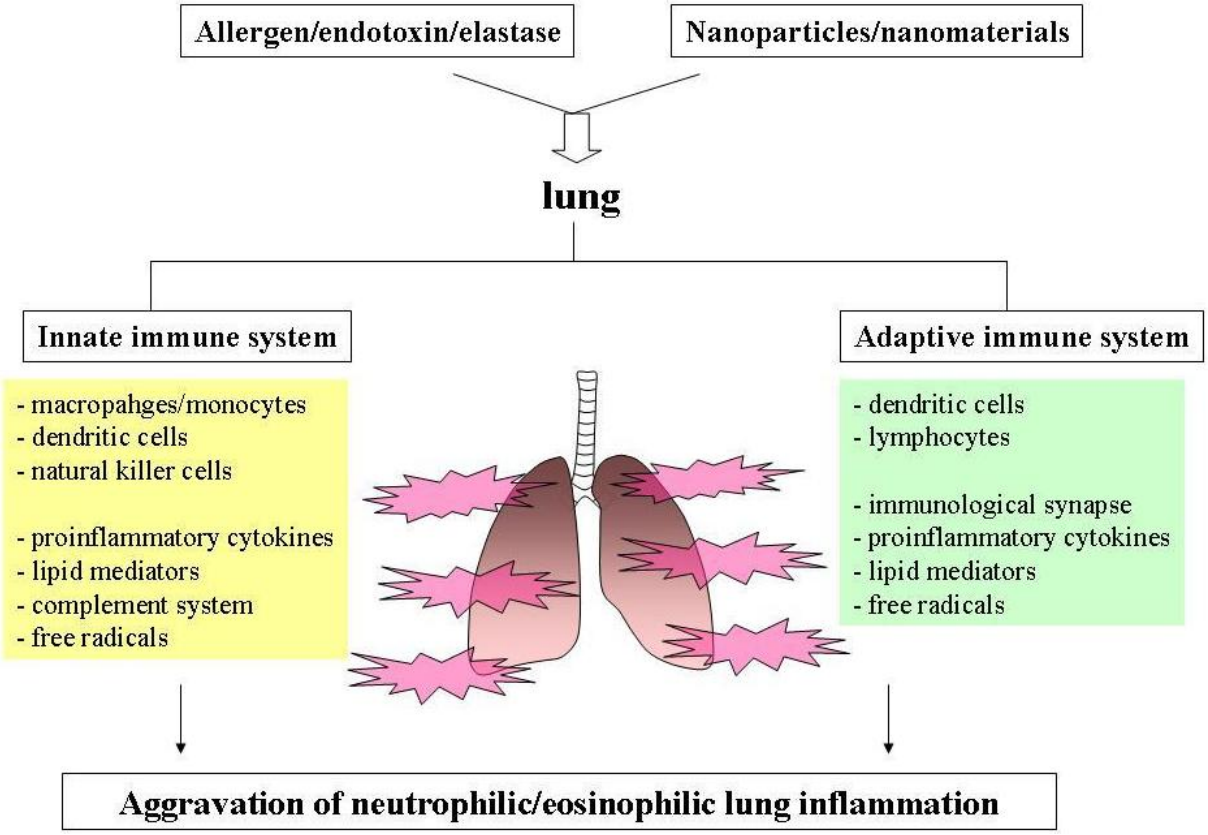

FIGURE 1. Proposal schema for aggravating effects of nanoparticles/nanomaterials on immune-mediated pulmonary inflammation. Regarding the impact on innate immunity, these particles may directly/indirectly influence on related cell populations, such as macrophages/monocytes, neutrophils, dendritic cells, natural killer cells, etc. As for adaptive immunity, in turn, nanoparticles/nanomaterials may potentially activate dendritic cells, lymphocytes, eosinophils, and mast cells/basophils. Furthermore, cell-cell interaction, intracellular signaling pathways, and/or chemical mediators, such as proinflammatory cytokines, complement system, and lipid mediators, may also be targets for these particles.

Vehicle, two sizes of CBNP, ovalbumin (OVA), and OVA + CBNP were administered intratracheally. The cellular profile of BAL fluid; lung histology; expression of cytokines, chemokines, and 8-OHdG; and immunoglobulin production were studied. CBNP with a diameter of 14 or 56 nm aggravated allergen-related airway inflammation characterized by the infiltration of eosinophils, neutrophils, and mononuclear cells, and by an increase in the number of goblet cells in the bronchial epithelium. CBNP with allergen increased protein levels of IL-5, IL-6, IL-13, eotaxin, MCP-1, and RANTES (regulated on activation and normal T cells expressed and secreted) in the lung as compared with allergen alone. The formation of 8-OHdG was moderately induced by CBNP or allergen alone, and was markedly enhanced by allergen plus CBNP as compared with CBNP or allergen alone. The aggravation was more prominent with 14-nm CBNP than with 56-nm CBNP in terms of the overall trend. CBNP with a diameter of 14 nm exhibited adjuvant activity for total IgE and antigen-specific IgG and IgE. CBNP can aggravate allergen-related airway inflammation and immunoglobulin production, which becomes more prominent with smaller particles. The enhancement may be mediated, at least partly, by the increased local expression of IL-5 and eotaxin, and also by the modulated expression of IL-13, RANTES, MCP-1, and IL-6[56]. Consistent with our study, de Haar and colleagues have previously shown that nanoparticles (14 and 29 nm) potently facilitate allergic airway inflammation as compared to fine particles (250 and 260 nm)[57].

In ongoing reports, CBNP alone or OVA alone moderately enhanced cholinergic airway reactivity, as assessed by total respiratory system resistance (R) and Newtonian resistance (R_n). All the parameters of lung responsiveness, such as R, compliance, elastance, R_n, tissue damping, and tissue elastance, were worse in the OVA + CBNP groups than in the vehicle group, the corresponding CBNP groups, or the OVA group. The lung mRNA level for mucin (Muc)5ac was significantly higher in the OVA group than in the vehicle group, and further increased in the OVA + CBNP groups than in the OVA or CBNP groups. These data suggest that CBNP can facilitate lung physiology, such as airway hyper-responsiveness, especially in the presence of allergen. Further, the effects may be mediated, at least partly, through the enhanced lung expression of Muc5ac[58].

We recently demonstrated that (single- and multiwalled) carbon nanotubes promote allergic airway inflammation in mice, which may be partly through enhanced oxidative stress in the airway and the inappropriate activation of antigen-presenting cells, including dendritic cells (*in vitro*)[59,60]. In addition, other groups have reported the similar impacts of nanoparticles (carbon nanotubes, TiO_2, gold) as we did on animal allergic asthma models[57,61,62,63]. Moreover, as for cellular contribution, we and others have claimed that antigen-presenting cells, such as dendritic cells, are important target cell populations for the adjuvant activity of nanoparticles[64,65,66]. Taken together, nanoparticle exposure can exacerbate allergic asthma (Fig. 1).

EFFECTS OF NANOPARTICLES ON ELASTASE-ELICITED PULMONARY INFLAMMATION

Chronic obstructive pulmonary disease (COPD) is one of the chronic inflammatory diseases of the lung that is associated with reduced maximal expiratory flow, increased lung volume, and alveolar wall destruction[67]. COPD is currently the fourth leading cause of death in the U.S., with up to 7 million patients diagnosed each year[68]. PM is epidemiologically implicated to link to the degree of symptoms of COPD[69,70,71]; however, biological evidence remains unclarified. We recently investigated the impact of pulmonary exposure to CBNP on COPD-like emphysematous lung injury induced by porcine pancreatic elastase (PPE) in mice. Vehicle, two sizes (14 and 56 nm) of CBNP, PPE, or PPE + CBNP was administered intratracheally; thereafter, parameters of inflammatory lung changes were evaluated at several time points. CBNP of 14 nm significantly induced acute lung inflammation in nonelicited subjects, and aggravated PPE-elicited airway inflammation with infiltrated neutrophils and eosinophils at an early stage, which was concomitant with the enhanced lung expression of proinflammatory cytokines related to innate immunity, such as IL-1β, and chemokines, such as KC. Further, 14-nm CBNP exaggerated emphysematous lung structural changes at a delayed stage. On the other hand, 56-nm CBNP induced lung inflammation, but did not influence PPE-elicited pathophysiologic traits in the lung. Taken together, CBNP at an optimal size and dose can exacerbate PPE-induced pulmonary inflammatory response and emphysema. This enhancement may be mediated, at least partly, via the increased local expression of proinflammatory molecules([72], and unpublished data) (Fig. 1).

SUMMARY

In summary, both airborne and engineered nanoparticles/nanomaterials can facilitate pathological pulmonary inflammation, sometimes with synergism, implying that particle size plays a critical role in determining the extent of these types of immune-mediated pulmonary inflammation. Regarding the impact on innate immunity, these particles may directly/indirectly influence on related cell populations, such as macrophages/monocytes, neutrophils, dendritic cells, natural killer cells, etc. As for adaptive immunity, in turn, nanoparticles/nanomaterials may potentially activate dendritic cells, lymphocytes, eosinophils, and mast cells/basophils. Furthermore, cell-cell interaction, intracellular signaling pathways, and/or chemical mediators, such as proinflammatory cytokines, complement system, and lipid mediators,

may also be targets for these particles (Fig. 1). Although it is easy to imagine that characteristics of the particles, such as physicochemical properties, electronic charge, aggregation rate, surface coating, colloidal stability, etc., are important for differences in the impact on immune modulation, future investigation warrants the matter. In any case, these previous findings indicate that nanoparticles/ nanomaterials potentiate an inflammatory response in subjects with lung inflammation, which might play a vital role in the pulmonary effects of airborne pollutants on the sensitive populations who have preceding respiratory immune-mediated diseases.

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